AD_____

Award Number: W81XWH-06-1-0289

TITLE: Scavenger Receptors and Resistance to Inhaled Allergens

PRINCIPAL INVESTIGATOR: Lester Kobzik, M.D.

CONTRACTING ORGANIZATION: Harvard School of Public Health
Boston, MA 02115

REPORT DATE: February 2008

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
16-02-2008	Annual	17 JAN 2007 - 16 JAN 2008

4. TITLE AND SUBTITLE
Scavenger Receptors and Resistance to Inhaled Allergens

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0289

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Lester Kobzik, M.D.

Email: lkobzik@hsph.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Harvard School of Public Health
Boston, MA 02115

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

After OVA sensitization and aerosol challenge, SR-AI/II and MARCO-deficient mice exhibited greater eosinophilic airway inflammation and airway hyperresponsiveness compared to wild-type mice. A role for simple SRA-mediated antigen clearance ('scavenging') by lung macrophages was excluded by observation of comparable uptake of fluorescent OVA by wild-type and SRA-deficient lung M√s and DCs. In contrast, airway instillation of fluorescent antigen revealed significantly higher traffic of labelled DCs to thoracic lymph nodes in SRA-deficient mice than in controls. The increased migration of SRA-deficient DCs was accompanied by enhanced proliferation in thoracic lymph nodes of adoptively transferred OVA-specific T cells after airway OVA challenge. The data identify a novel role for SRAs expressed on lung DCs in down-regulation of specific immune responses to aero-allergens by reduction of DC migration from the site of antigen uptake to the draining lymph nodes.

15. SUBJECT TERMS

asthma, allergy, scavenger receptors, dendritic cells

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	6	USAMRMC

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Our central hypothesis is that lung macrophage scavenger receptors normally function to bind and clear inhaled allergens and pathogens, thereby preventing allergic responses and infections. The purpose of the project is to determine whether 1) decreased levels of SRAs (mediated by environmental stresses) increase susceptibility to asthma or pneumonia; and 2) therapy to increase or maintain normal levels of scavenger receptors will increase resistance to asthma and pneumonia. The scope of the research includes studies using in vivo mouse models (Aim 1), studies of the specific role of alveolar macrophages (Aim 2) and dendritic cells (Aim 3) and studies of the effects of pollutants on scavenger receptors (Aim 4).

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement of Work.

For this period, our SOW identified two main tasks to be started:

Task 2: **Determine role of SRAs on DCs in responses to inhaled allergen:** This work will test the hypothesis that DC SRAs act to down-regulate allergic immune responses. (Mos. 12-36):

- compare phenotype (cell surface markers) and function (migration, T cell stimulation, antigen uptake, signaling by SRA cross-linking) of DCs from wild-type (WT) and KO mice in vivo and in vitro.
- use DC deletion and adoptive re-constitution with wild-type or KO DCs to specifically test their ability to modulate responses.

Task 3: **Determine role of SRAs on AMs in responses to inhaled allergen:** This work will test the hypothesis that AM SRAs also mediate down-regulation of immune responses to allergens.
(Mos. 12-36):

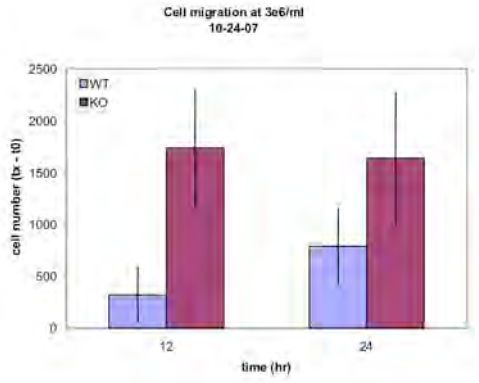
- use adoptive transfer of wild-type or KO AMs to specifically test their roles in modulation of allergic responses in vivo
- use in vitro assays with wildtype or KO AMs to test their modulation of DC functions by nitric oxide (NO) or other candidate molecules
-

In vitro component of Task 2 & 3: We are please to report good progress in Task 2. To study dendritic cells we have learned and optimized in vitro culture protocols to grow bone-marrow derived dendritic cells from both wild-type and SRA knockout mice. This produces a large number of cells suitable for easy analysis of phenotype and function. We have confirmed purity of the cultured dendritic cells by immunolabeling with CD11c marker (>95% +). Functional assays to compare DCs from wild-type and knockout mice have started. For example, to test whether scavenger receptors reduce cell motility (as observed in vivo as increased DC accumulation in lymph nodes in our asthma model), we have developed microscopic and live cell imaging assays to quantitate cell

movement. The first assay uses the Oris migration chambers and results in counting of cells that move from an upper chamber to the lower chamber. As illustrated in the figure, initial results are promising and show increased migration of MARCO deficient DCs. The second assay measures random migration by tracing cell movement over 12-24 hours in a live cell system. We have completed optimization of this assay with wild-type cells and willl compare outcomes in knockout cells next.

Additional functional assays are in progress now that we have an abundant supply of DCs, which facilitates replicates and dose-response analyses.

Increased migration in MARCO deficient dendritic Cells:



In vivo component of task 2& 3. Testing the effect of adoptive transfer of SRA-expressing DCs or AMs into the lungs of knockout mice is the key experiment in vivo in these two aims. We have devoted considerable effort this year to generate powerful tools to optimize and enhance these experiments. Namely, we have developed highly efficient lentiviral constructs to allow robust expression of MARCO (and SRA) in transduced knockout macrophages and DCs. We have worked in collaboration with Dr. Igor Kramnik, another faculty member at HSPH, who has developed a 'lentiviral tool box' for mouse macrophages and DCs , with plasmids that allow easy selection and high levels of expression. We have engineered the appropriate plasmids with full-length MARCO or SRA (as well as some truncation variants to dissect which components of the receptor are functional), including some vectors that feature inducible expression using doxycycline. These lentiviral constructs have now been correctly packed and purified and validated using mouse macrophages. We have confirmed robust expression of MARCO and SRA on the surface of transduced cells. These lentiviruses will be used to transduce high levels of expression into knockout DCs and macrophages, followed by adoptive transfer as outlined in the original proposal. These experiments are now set to begin, now that we have established this powerful technology. We have also begun the experiments with in vivo deletion of DCs to allow testing of this manipulation. These assays use diptheria toxin to eliminate DCs in transgenic mice, and we are validating the efficacy of this treatment prior to use of the model in the allergy studies and before adoptive transfer experiments.

KEY RESEARCH ACCOMPLISHMENTS:

- Established culture system to produce wild-type and knockout bone-marrow derived dendritic cells and macrophages
- Optimized cell migration assays and initiated these studies
- Engineered lentiviral constructs for high-level expression of MARCO and SRA in knockout dendritic cells and macrophages prior to adoptive transfer in vivo

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

1. Manuscript published in J. Immunology 2007; vol. 178: p.5912 (reported in last year's progress report)

CONCLUSION:

We have made good progress in both tasks 2 and 3 which span this past and coming year in the overall plan and SOW. The work in the past year has mostly developed new systems and tools to better complete the actual comparisons of knockout and wild-type cell functions. These have already started but will be the focus of activities in the coming year.

REFERENCES:

APPENDICES: